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# **Ventilatory responses to muscle metaboreflex activation in Chronic Obstructive Pulmonary Disease.**

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Running title: Ventilation and muscle metaboreflex in COPD.

## **Key points summary**

- Recent evidence indicates a role for group III/IV muscle afferents in reflex control of the human ventilatory response to exercise.
- Dyspnoea in chronic obstructive pulmonary disease (COPD) may be linked to this reflex response.
- This study shows that activation of the muscle metaboreflex causes a ventilatory response in COPD patients but not in healthy controls.
- This indicates abnormal involvement of muscle afferents in the control of ventilation in COPD which may be a contributing factor to exercise dyspnoea.

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## **Abstract.**

Blockade of thin fibre muscle afferent feedback during dynamic exercise reduces exercise hyperpnoea in health and chronic obstructive pulmonary disease (COPD). Therefore, we hypothesised that activation of the muscle metaboreflex at rest would cause hyperpnoea. We evaluated the effect of muscle metaboreflex activation on ventilation, in resting COPD patients and healthy participants. Following a bout of rhythmic hand grip exercise, post exercise circulatory occlusion (PECO) was applied to the resting forearm to sustain activation of the muscle metaboreflex, in 18 COPD patients (FEV<sub>1</sub>/FVC ratio <70%), 9 also classified as chronically hypercapnic, and 9 age and gender matched controls. The cardiovascular response to exercise and the sustained blood pressure elevation during PECO was similar in patients and controls. During exercise ventilation increased by  $6.64 \pm 0.84$  in controls and significantly ( $p < 0.05$ ) more,  $8.38 \pm 0.81$  l.min<sup>-1</sup> in patients. During PECO it fell to baseline levels in controls but remained significantly ( $p < 0.05$ ) elevated by  $2.78 \pm 0.51$  l.min<sup>-1</sup> in patients until release of circulatory occlusion, with no significant difference in responses between patient groups. Muscle metaboreflex activation causes increased ventilation in COPD patients but not in healthy participants. Chronic hypercapnia in COPD patients does not exaggerate this response. The muscle metaboreflex appears to be abnormally involved in the control of ventilation in COPD and may be a contributing factor to exercise dyspnoea.

## **Abbreviations list.**

COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure;  $f$ , respiratory frequency; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; HCO<sub>3</sub><sup>-</sup>, bicarbonate; HF, heart failure; HR, heart rate; MAP, mean arterial blood pressure; MVC, maximal voluntary contraction; PaCO<sub>2</sub>, partial pressure of arterial carbon dioxide; PaO<sub>2</sub>, partial pressure of arterial oxygen; PECO, post exercise circulatory occlusion;  $P_{ET}CO_2$ , partial pressure of end tidal carbon dioxide; SBP, systolic blood pressure;  $V$ , minute ventilation.

## Introduction

It is well established that cardiovascular responses to exercise are controlled in part by central command (Goodwin et al 1972) and partly by metabolically and mechanically sensitive thin fibre afferents (groups III and IV) arising from the exercising skeletal muscle (Coote et al. 1971, McCloskey & Mitchell 1972). Selective experimental stimulation of metaboreceptive afferents is commonly achieved via the technique of post exercise circulatory occlusion (PECO). In this technique, the occlusion of blood flow immediately post exercise traps metabolites including; ATP, lactic acid/H<sup>+</sup>, bradykinin, arachidonic acid and its cyclo-oxygenase products (Kaufman *et al.* 1983, Rotto & Kaufman, 1988, Hanna & Kaufman, 2004) within the previously exercising muscle and so stimulates metabosensitive fibres. It has been consistently shown that stimulation of these afferents via PECO induces an increased activation of the sympathetic nervous system and a resultant pressor reflex (Alam & Smirk 1937, Seals et al. 1988).

Whilst the role of central command in driving exercise hyperpnoea is also well established (Krogh and Lindhard 1913, Eldridge et al 1981, Green et al 2007) the role of skeletal muscle afferent feedback in controlling the exercise hyperpnoea is more controversial. Traditionally, it has been considered unimportant as evidenced by the classic finding in healthy humans that PECO does not prevent the recovery of ventilation to resting levels (Rowell et al. 1976, Innes et al. 1989, Haouzi et al. 2001). However, recent evidence, involving the blockade of afferent feedback during dynamic exercise has revealed a contribution of muscle afferents to ventilatory drive (Amann et al 2010). Intrathecal administration of Fentanyl reduced exercise hyperpnoea in both healthy participants, chronic obstructive pulmonary disease (COPD) and heart failure (HF) patient groups (Gagnon et al 2012, Olson et al 2014). Additionally, we have shown that in healthy participants activation of muscle afferents using PECO, combined with mild hypercapnia, generates a ventilatory response and there is evidence of a synergistic interaction between central chemoreflex activation and muscle afferent feedback (Lykidis et al. 2010; Bruce & White 2012, 2015).

Patients with COPD commonly develop skeletal muscle dysfunction; both in terms of a decreased force generating capacity and derangements in energy metabolism. There is an increasing body of evidence suggesting that, alongside the respiratory impairments, skeletal muscle dysfunction is an important contributing factor in the exercise intolerance associated with the disease (see Maltais et al. 2014). A slow to fast transition of muscle fibre types

(Whittom *et al.* 1998; Jobin *et al.* 1998; Gosker *et al.* 2002), increased muscle glycolytic enzyme activity (Jakobsson *et al.* 1995; Gea *et al.* 2001), and reduced mitochondrial density and (accordingly) oxidative enzyme activity (Jakobsson *et al.* 1995; Gosker *et al.* 2002, 2007) have been demonstrated. These maladaptations may be important determinants in reducing fatigue resistance commonly found in COPD.

During exercise the increased glycolytic capacity and shift away from oxidative metabolism results in a greater and earlier onset muscle acidosis. (see Puente-Maestu *et al.* 2013, for review). This would be expected to generate an augmented metaboreflex, and if muscle afferent feedback does provide a drive to ventilation, it could contribute to dyspnoea and exercise intolerance. However, to our knowledge, no controlled studies have yet examined the ventilation of COPD patients during metaboreflex activation using PECO. Therefore the primary aim of this investigation is to examine the respiratory responses of patients with COPD to PECO, compared with age matched healthy control participants. We hypothesise that patients with COPD will demonstrate augmented ventilatory responses to metaboreflex activation.

Secondly, based on our previous observations of a synergistic interaction between experimentally induced hypercapnia and metaboreflex activation, we aim to examine whether any respiratory response to PECO in COPD patients relates to their level of CO<sub>2</sub> retention. (Lykidis *et al.* 2010; Bruce & White 2012, 2015). We hypothesise that chronically hypercapnic patients will generate a larger ventilatory response during PECO in comparison to normocapnic patients.

## **Methods**

### **Ethical Approval**

All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. All subjects were habituated to all the experimental procedures, which conform to the declaration of Helsinki and were approved by an NHS ethical committee.

## Participants

18 patients with stable moderate to severe COPD, defined according to Global Initiative for Obstructive Lung Disease criteria, were recruited. Patient pulmonary function data and participant characteristics are shown in Table 1. COPD was defined as FEV<sub>1</sub>/FVC ratio <70% following bronchodilator medication. Moderate COPD was defined as a FEV<sub>1</sub>% predicted between 50%-79% and severe COPD between 30%-49%. The medications prescribed to the patients are listed in Table 2. Patients were not considered stable and excluded from participation if in the previous 6 weeks they had been hospitalised or suffered an exacerbation or infection. Patients with heart failure/disease, type II diabetes, renal disease or an active malignancy revealed by medical history were also excluded. In total, 4 COPD patients had controlled hypertension managed by the prescribed antihypertensive medications also listed in Table 2. All patients continued to adhere to their prescribed drug regimens throughout the study. 9 healthy age and gender matched controls also volunteered for this study. Participants visited the laboratory for one trial day following habituation. Before the trial day participants refrained from consuming food and caffeine in the 4 hours before the trial and from performing strenuous physical activity or consuming alcohol in the 12 hours before the trial. This study followed a within subject design with all subjects participating in both trials. The order of each 8 minute trial was randomised with a 30 minutes rest period in between.

## Experimental procedures

Participants were seated in an upright position, held a custom made handgrip dynamometer with their right hand. The device was clamped to a surface top which also supported the participant's right arm. Maximal voluntary contractions (MVC) were determined by instructing participants to perform 3-5 maximal handgrip efforts separated by one minute and the highest was taken as the MVC. During the experimental protocol participants maintained the required percentage of their MVC by matching their force output to a target force displayed on a computer screen positioned in front of them at eye level. The rhythmic isometric handgrip exercise task was performed at a duty cycle of 1 second contraction at 50% MVC, to 1 second relaxation, and the rhythm was maintained via a metronome.

The experimental protocol is shown in Figure 1. Prior to the start of each trial participants rested for 5 minutes in order to establish steady state ventilation and cardiovascular variables.

Both trials then consisted of a 2 minute baseline recording period before a 2 minute rhythmic isometric handgrip exercise task. Participants then either:

- 1) Rested for a further 4 minutes (**Control trial**) or
- 2) A cuff was rapidly inflated to 200mmHg around the upper right arm by a rapid cuff inflator commencing 2-3 contractions before cessation of the exercise period ensuring that immediately after exercise circulatory occlusion was achieved and so participants went through a 2 minute period of PECO. Then the cuff was deflated and participants rested for 2 minutes (**PECO trial**)

The order of each 8 minute trial was randomised with a 30 minutes rest period in between.

#### Respiratory and Cardiovascular measures

Ventilation was continuously monitored with a pneumotachograph (Flowmetrics, BRDL, fr-41 s, ultasonic pneumotachograph, CA, USA) attached to the inspiratory side of a breathing valve (T-Valve) with a mouthpiece. From this the respiratory frequency ( $f$ ) and inspiratory airflows (L/sec) were measured and so minute ventilation ( $V$ ) was calculated. All volumes recorded were converted to BTPS. The pneumotachograph was calibrated with a 3 litre syringe to give a linear output over the range of 0.2 L to 3 L. The partial pressure of end tidal  $\text{CO}_2$  ( $P_{\text{ETCO}_2}$ ) was recorded throughout the protocol with a rapid gas analyser (Servomex, 1440, Sussex, UK) sampling the end tidal gases in the breathing valve. The analyser was calibrated using gases with a known concentration of  $\text{CO}_2$  (between 0% and 10%). Heart rate (HR) was derived from a 3 lead electrocardiogram (ECG; Cardiorator CR7, Cardiac Records Ltd, London, UK) in the lead II position. From this, heart rate (HR) was continuously measured. Blood pressure was measured using finger photoplethysmography (Portapress, Finapres Medical Systems, Amsterdam, The Netherlands) with the cuff placed on the middle finger, and the hand supported at heart level on an adjustable table.

#### Data analysis

Mean averages for  $V$ ,  $f$ ,  $P_{\text{ETCO}_2}$ , HR and mean arterial pressure (MAP) during each minute of the trials were calculated. The cardiovascular and ventilatory responses to exercise and PECO were assessed with an analysis of variance with repeated measures, and then when appropriate multiple comparison *post hoc* analysis. Comparisons between the control participants and COPD patients in both trials were assessed using an unpaired Student's  $t$ -test. Data are expressed as mean  $\pm$  SEM and statistical significance was taken as ( $P < 0.05$ ).

Statistical analysis was conducted using a standard statistical package (SPSS, Chicago, IL, USA).

## **Results**

### Participant characteristics

Participant characteristics are presented in Table 1. In respect of age, resting cardiovascular variables and body size there were no significant differences between healthy participants and the patients. However, COPD patients produced a 17% lower handgrip force than healthy participants ( $P < 0.05$ ).

### Differences between trials at baseline

The mean  $\dot{V}$ ,  $f$ , HR, MAP and  $P_{ET}CO_2$  during baseline are presented in Table 3. There were no significant differences in any of these variables between the Control and COPD participants.

### Respiratory responses

Changes in mean  $\dot{V}$  relative to baseline recorded during each of the 4 trials are shown in figures 2A and 2B. Exercise caused significant increases in  $\dot{V}$  during the first and second minute of exercise in all trials. During the second minute of exercise, the  $\dot{V}$  of healthy participants increased by  $6.36 \pm 0.79 \text{ l.min}^{-1}$  and  $6.64 \pm 0.84 \text{ l.min}^{-1}$  in the control and PECO trials respectively. In the COPD patients  $\dot{V}$  increased by  $8.1 \pm 0.64 \text{ l.min}^{-1}$  and  $8.38 \pm 0.81 \text{ l.min}^{-1}$  in control and PECO trials respectively, with higher  $\dot{V}$  response in the final 30 seconds of exercise in patients compared to the healthy participants ( $p < 0.05$ ).  $\dot{V}$  returned to baseline levels in both the Healthy Control and Healthy PECO trials following exercise.  $\dot{V}$  remained significantly above baseline during the first minute after exercise in the COPD Control trial ( $2.23 \pm 0.33 \text{ l.min}^{-1}$ ) but returned to baseline in the second minute. During the COPD PECO trial,  $\dot{V}$  remained significantly elevated above baseline throughout PECO ( $3.82 \pm 0.68 \text{ l.min}^{-1}$  and  $2.78 \pm 0.51 \text{ l.min}^{-1}$ ) only returning to baseline after cuff deflation.  $\dot{V}$  during PECO in the COPD PECO trial was significantly higher than that in the Healthy PECO trial. During the recovery period,  $\dot{V}$  was not significantly different from baseline in all 4 trials ( $P > 0.05$ ).



The increased ventilation during exercise was related to change in both tidal volume and  $f$ , however, only the increases in  $f$  reached statistical significance, during the last minute of exercise, in all trials. In the COPD patients these increases were significantly ( $P<0.05$ ) greater in comparison to the healthy participants in the control ( $10.6 \pm 3.1$  and  $6.7 \pm 2.2$  breaths.min<sup>-1</sup>) and PECO trials ( $11.6 \pm 2.8$  and  $7.2 \pm 2.9$  breaths.min<sup>-1</sup>). Following exercise, during the last minute of rest in the control trials,  $f$  recovered to baseline levels in both groups ( $1.9 \pm 3.3$  and  $0.4 \pm 1.9$  breaths.min<sup>-1</sup>). In the PECO trials  $f$  remained significantly elevated above baseline levels in the COPD patients in contrast to the recovery to baseline levels seen in the healthy participants ( $4.9 \pm 2.5$  and  $2.1 \pm 2.2$  breaths.min<sup>-1</sup>).

Figure 2C and 2D shows the mean  $P_{ET}CO_2$  values during throughout the 4 trials. There was no significant change in  $P_{ET}CO_2$  from baseline levels within each trial and no difference between the trials.

#### Cardiovascular responses

Changes in mean HR and MAP relative to baseline during the 4 trials are shown in figure 2E-H. Both MAP and HR significantly increased during exercise in the Healthy Control trial ( $13 \pm 2.3$  mmHg and  $12 \pm 1.5$  beats.min<sup>-1</sup>;  $P<0.05$  versus baseline), in the Healthy PECO trial ( $13 \pm 1.6$  mmHg and  $14 \pm 1$ ;  $P<0.05$  versus baseline), in the COPD Control trial ( $14 \pm 1.1$  mmHg and  $13 \pm 1$  beats.min<sup>-1</sup>;  $P<0.05$  versus baseline) and in the COPD PECO trial ( $13 \pm 1$  mmHg and  $12 \pm 1.2$ ;  $P<0.05$  versus baseline). MAP and HR returned to baseline levels after exercise in the Healthy Control and COPD Control trials. However in both the PECO trials MAP fell from exercise levels but remained significantly elevated above baseline during the 2 minutes post exercise ( $P<0.05$ ) and only returned to baseline after cuff deflation. There were no significant differences in the blood pressure responses during PECO in both the Healthy PECO and COPD PECO trials. During the recovery period, both MAP and HR values were not significantly different from baseline in all 4 trials ( $P>0.05$ ).

#### Hypercapnic vs. Normocapnic COPD patients

COPD patients were separated into Hypercapnic patients ( $PaCO_2 > 45$ mmHg) and normocapnic patients. Table 4 shows that participants in the hypercapnic group had a significantly higher resting  $PaCO_2$ , arterial bicarbonate content and lower  $PaO_2$  values than those in the normocapnic group. No other significant differences between groups were observed.

### Respiratory and cardiovascular responses during the PECO trial

Baseline  $V$ ,  $f$ ,  $P_{ET}CO_2$ , HR and MAP in hypercapnic and normocapnic COPD patients during baseline of the PECO trials are shown in Table 5. As expected the  $P_{ET}CO_2$  of hypercapnic patients was significantly higher than normocapnic patients during baseline ( $+15 \pm 1.3$  mmHg,  $P<0.05$ ) and throughout the trial (Figure 3B). There were no significant differences between any other baseline variables.

Changes in mean  $V$  relative to baseline in hypercapnic and normocapnic COPD patients during each minute of the PECO trial are shown in figure 3A. No significant differences in  $V$  were observed between the hypercapnic and normocapnic patients at any time point in the trial. Changes in mean HR and MAP relative to baseline in hypercapnic and normocapnic COPD patients during each minute of the PECO trial are shown in figure 3C and 3D. No significant differences in HR and MAP were observed between the hypercapnic and normocapnic patients at any time point in the trial.

### **Discussion**

This study investigated the ventilatory and cardiovascular responses of patients with moderate-severe COPD and healthy aged matched controls, to rhythmic handgrip exercise and activation of the muscle metaboreflex. The main findings are that activation of the metaboreflex via PECO maintained ventilation significantly above baseline in COPD patients but not in the healthy controls. However, there was no difference in this response between hypercapnic and normocapnic COPD patients.

During PECO, where volition and muscle force are absent, only the activity of the muscle metaboreflex remains increased. The magnitude of the blood pressure response generated during PECO is known to be a reliable indicator of the level of muscle metaboreflex activation (Coote *et al.* 1971, McCloskey & Mitchell 1972, Kaufman *et al.* 1983). Therefore, our observation of similar blood pressure elevation during PECO in the patients and healthy participants is good evidence of a comparable level of muscle metaboreflex activation in both groups.

As expected, using PECO to maintain metaboreflex activation in isolation from central command and muscle mechanoreceptor activation, failed to sustain the ventilation of healthy participants above baseline values. This finding has been well documented in many studies of healthy individuals using circulatory occlusion following prior exercise e.g. handgrip and cycling exercise (Rowell *et al.* 1976, Innes *et al.* 1989, Haouzi *et al.* 2001,). This evidence forms a major component of the classical view that in humans, muscle afferent activation does not contribute to respiratory drive in exercise.

However, in our patients we demonstrated, for the first time, a sustained elevation of ventilation during PECO, which suggests that activation of the muscle metaboreflex can generate and maintain a respiratory response following forearm exercise. This is further supported by the finding that ventilation only returned to baseline values post arm-cuff deflation and thus removal of metaboreflex activation. Our observation of a ventilatory response, largely driven by increased  $f$ , to *activation* of the muscle metaboreflex is consistent with other recent evidence based upon *removal* of afferent input during exercise. Amann *et al.* (2010) showed that the hyperpnoea to dynamic cycling exercise can be attenuated by inhibiting the neurotransmission of group III and IV afferents from the exercising muscles via intrathecal administration of fentanyl. In addition, administration of this spinal anaesthesia to patients with COPD (Gagnon *et al.* 2012) and HF (Olson *et al.* 2014) reduced the exercise ventilatory response,  $f$  and sensations of dyspnoea, while enhancing cycling exercise endurance time. When considered together this evidence based on both removal and addition of muscle afferent feedback, reveals a potentially important role for it in human respiratory control. In addition, whereas Gagnon *et al.* demonstrated that muscle afferent feedback plays an important role in the control of the exercise hyperpnoea and may limit exercise capacity in COPD, our methodology has the advantage of showing that the sensitivity of the muscle afferent mediated control of ventilation may be increased in COPD. Furthermore, our use of PECO selectively activates the muscle metaboreflex and so clearly defines its contribution in the absence of muscle mechanoreceptor input.

A disproportionate exercise ventilatory response in COPD is well documented and indeed our patients did produce a significantly greater ventilatory response during the rhythmic handgrip exercise task compared with healthy aged matched controls. As these patients also displayed an augmented ventilatory response to PECO compared with healthy controls our results suggest that metaboreflex activation may contribute to this characteristic excessive exercise ventilatory response in COPD. This contribution could be mediated by two mechanisms.

First, a greater local metabolite accumulation in the muscle causing enhanced activation of the muscle metaboreflex. Second, there could be a greater central sensitivity to the same level of muscle afferent input.

The first explanation is plausible as COPD is associated with the down regulation of skeletal muscle oxidative enzyme activity, an increased lactic acidosis and a lower muscle pH in exercise (Casaburi *et al.* 1991 Kutsuzawa *et al.* 1992, Gosker *et al.* 2002). Indeed Kutsuzawa *et al.* found an exaggerated decrease in the pH of forearm muscles during a standardized handgrip exercise in patients with COPD compared with healthy controls. Animal studies show that the arterial injection of lactic acid/H<sup>+</sup> causes an increase in the discharge of group III and IV muscle afferent fibres (Rotto & Kaufman 1988) and an increase in ventilation and blood pressure (Rotto *et al.* 1989). This is likely mediated, in part, by the stimulation of acid sensing ion channels located on the free nerve endings of muscle afferent fibres (Li *et al.* 2004; Hayes *et al.* 2008). Therefore the skeletal muscle dysfunction associated with COPD may result in a standard exercise bout producing increased stimulation of metabosensitive receptors, an augmented metaboreflex, and so an exaggerated ventilatory response.

However, the blood pressure response of the COPD patients to PECO was not significantly different to that of healthy controls, a finding consistent with other reports using static handgrip exercise (Roseguini *et al.* 2008 and Sherman *et al.* 2011). This is consistent with a similar level of muscle afferent activation between patients and healthy controls. If so, then our second explanation for an augmented ventilatory response to PECO, becomes more appealing. Indeed, in healthy participants, we have shown that it is possible to manipulate acutely, the sensitivity of the respiratory control system to muscle afferent input (Lykidis *et al.* 2010; Bruce & White, 2012). These studies used exposure to mild hypercapnia to alter the level of central respiratory activity in combination with a controlled level of muscle afferent activation. Under conditions of mild hypercapnia, muscle metaboreflex activation using PECO generated a normal pressor response. However, the elevation of ventilation, caused by prior exercise was sustained, in a manner resembling the response of the COPD patients in the present study. This prior work suggests a differential response by central cardiovascular and respiratory control networks to muscle metaboreflex activation, and the present study supports this view.

In additional experiments this hyperventilation, during PECO, was shown to be unrelated to exposure of the active muscle to hypercapnia and it was not attenuated by acute exposure to

hyperoxia, thereby reducing peripheral chemoreflex stimulation (Bruce and White, 2015). These observations have been argued to be evidence of a synergistic interaction between the central chemoreflex and muscle afferent input (Coote 2012). This was the rationale for comparing hypercapnic COPD patients, normocapnic COPD patients and healthy control participants. Knowing that some COPD patients retain CO<sub>2</sub>, we hypothesised that such patients might display an exaggerated response to PECO. However, we found no differences in the ventilatory responses to PECO between groups of hypercapnic and normocapnic COPD patients. It appears unlikely that the level of muscle metaboreflex differed between the two groups as their blood pressure responses to PECO were similar. So, the explanation for these findings may relate to differences between chronic exposure and acute exposure to hypercapnia. It is known that chronic elevation of PaCO<sub>2</sub> causes desensitisation of central chemoreceptors and this is classically found in CO<sub>2</sub> retaining COPD patients (Richerson and Boron, 2005) but further studies are required to examine this mechanism more fully.

### Implications

Physical inactivity is considered an important factor in the development of skeletal muscle dysfunction in COPD (Serres *et al.* 1998) playing a part in a “dyspnoea spiral” (Prefaut *et al.*, 1995). Patients avoid physical exertion to prevent sensations of dyspnoea, resulting in further deconditioning of skeletal muscles. A downward spiral may result, further exaggerating the exercise ventilatory response and exacerbating the sensations of dyspnoea in exercise with further deterioration in exercise tolerance over time. This is analogous to the “muscle hypothesis” used to explain effort intolerance in HF (Coats *et al.* 1994), a patient group who share similar features of skeletal muscle dysfunction to COPD (Franssen *et al.* 2002), and produce augmented respiratory and cardiovascular responses to PECO (Piepoli *et al.* 1996, 1999; Scott *et al.* 2002; Ponikowski *et al.* 2001; Notarius *et al.* 2001; Crisafulli *et al.* 2007; Olson *et al.* 2010). Regardless of whether afferent feedback is increased in COPD patients, our results show for the first time, an exaggerated respiratory response to metaboreflex activation in these patients, providing new insight into the neural link between skeletal muscle afferent activation and exercise dyspnoea in COPD.

Endurance training in COPD patients has been shown to improve the metabolic efficiency of skeletal muscle by increasing oxidative enzyme activities and reducing lactic acid production and skeletal muscle acidosis in exercise (Casaburi *et al.* 1991; Maltias *et al.* 1996; Whittom *et al.* 1998; Sala *et al.* 1999). Training of healthy subjects certainly results in attenuated muscle metaboreflex mediated changes in muscle sympathetic nerve activity (Sinoway *et al.* 1989)

and blood pressure (Fisher and White, 1999). An improvement in skeletal muscle oxidative capacity and reduced muscle acidosis in exercise may therefore attenuate the stimulation of metabolically sensitive receptors in skeletal muscle and thus reduce the central respiratory drive and ventilatory response to the exercise. As such, the sensations of dyspnoea upon exertion may be attenuated and exercise tolerance may increase. Indeed a 6 week training programme of the forearm muscles in HF has shown to reduce the ventilatory and cardiovascular responses to PECO following rhythmic handgrip exercise (Piepoli *et al.* 1996), and this was thought to be achieved through improvements in skeletal muscle metabolism and the reduced stimulation of the muscle metaboreflex (Piepoli *et al.* 2008). However similar studies evaluating muscle afferent adaptation to training are yet to be conducted in COPD patients.

### Limitations

Although the primary objective of this study was to examine ventilatory responses to activation of the muscle metaboreflex, we evaluated the level of this reflex activation by measurement of the pressor response to PECO. Recording of muscle sympathetic nerve activity would provide a more direct estimation of the efferent outflow resulting from muscle metaboreflex activation though this still would not be a direct measure of the muscle afferent activity which remains impractical in human studies. The standardization of exercise conditions also becomes an important issue in respect of comparing responses in groups with different muscle strengths. In the present study the MVC's of the patient group were on average 17% lower. Though they performed the same relative exercise intensity as the healthy controls (50% of MVC) we cannot be certain that this rhythmic isometric force x time integral produced the same metabolite accumulations during the crucial PECO phase of the experiments. If lower metabolite accumulation resulted in the patient group, due to lower absolute force production, then the similar pressor response to healthy participants might suggest an exaggerated cardiovascular response to metaboreceptor activation. However, the literature pertaining to sustained isometric contractions is clear that the exercise pressor response is unrelated to muscle strength under well controlled conditions (Fisher and White, 2004)

In our previous studies we had access to an in-house dynamic end tidal forcing system to clamp  $p_{ET}CO_2$  at a given level. Constraints of ethical approval meant that the present study was performed in an off site, hospital environment where we were unable to transport this

system. As a result, in the COPD patient trials where hyperventilation during PECO was observed there was a slight (non-significant) fall in  $P_{ET}CO_2$ . However, if anything, this would be expected to cause a small underestimate of the influence of muscle metaboreflex on ventilation in the COPD patients. Though set against this, the known desensitization of chemoreception in COPD patients makes its influence less likely. We also acknowledge that the study of ventilatory responses to muscle metaboreflex activation in the forearm muscles should be extended to study of the functionally more significant larger locomotor muscle masses of the legs.

### Conclusion

In conclusion this study demonstrated that the activation of the metaboreflex via PECO increases ventilation significantly above baseline in COPD patients but not in healthy controls. However, these ventilatory responses to PECO were not related to  $PaCO_2$ . The findings of this study further define the role of skeletal muscle afferent feedback in the augmented ventilation and sensations of dyspnoea that are associated with COPD during exercise. The interaction of muscle afferent feedback with other drivers of ventilation require further study.

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## **Additional information**

### Competing interests

The authors declare they have no competing interests

### Author contributions

RMB, AT and MJW conceived and designed the research. RMB performed the experiments and analysed the data. RMB, AT and MJW interpreted the results of the experiments and drafted the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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### Table legends.

Table 1. Participant characteristics ( $\pm$  SD). \* Significant difference from Healthy participants ( $P<0.05$ ). FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; HCO<sub>3</sub><sup>-</sup>, arterial bicarbonate content.

Table 2. Mean V, HR, MAP, and P<sub>ET</sub>CO<sub>2</sub> values recorded during the two minute baseline period in the Control and PECO trials for the Healthy and COPD patients.

Table 3 Characteristics of patients ( $\pm$ SD) separated into hypercapnic and normocapnic groups. \* Significant difference from Normocapnic participants ( $P<0.05$ ). FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; HCO<sub>3</sub><sup>-</sup>, arterial bicarbonate content.

Table 4. Mean V, HR, MAP, and P<sub>ET</sub>CO<sub>2</sub> values recorded during the two minute baseline period in the PECO trials of the normocapnic and hypercapnic COPD patients. \* Significant difference from normocapnia ( $P<0.05$ )

**Table 1.**

	Healthy	COPD
N	9 (5 male)	18 (10 male)
Age	67.7 ± 6.1	65.1 ± 5.1
Height (cm)	167.1 ± 4.9	162.2 ± 3.2
Weight (Kg)	71.8 ± 3.7	75.6 ± 3.1
Handgrip Force (N)	298 ± 45	246 ± 37*
Resting SBP (mmHg)	125 ± 3.2	119 ± 2.4
Resting DBP (mmHg)	78 ± 2.6	75 ± 1.9
Resting MAP (mmHg)	93 ± 3.1	89 ± 1.6
Resting HR (bpm)	71 ± 1.6	68 ± 1.3
FEV <sub>1</sub> (L)	-	1.06 ± 0.3
FEV <sub>1</sub> (% predicted)	-	42 ± 5.7
FVC (L)	-	2.35 ± 0.9
FEV <sub>1</sub> /FVC (%)	-	45 ± 3.4
PaO <sub>2</sub> (mmHg)	-	67.4 ± 9.2
PaCO <sub>2</sub> (mmHg)	-	43.4 ± 6.5
pH	-	7.41 ± 0.03
HCO <sub>3</sub> <sup>-</sup> (mEq.L <sup>-1</sup> )	-	27.8 ± 3.1

**Table 2.**

	<b>Trial</b>	<b>V (l.min<sup>-1</sup>)</b>	<b>HR (beats.min<sup>-1</sup>)</b>	<b>MAP (mmHg)</b>	<b>P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>
<b>Healthy</b>	<b>Control</b>	8.72 ± 0.92	68 ± 1.3	93 ± 3.1	39 ± 1.6
	<b>PECO</b>	9.09 ± 1.02	69 ± 1.4	95 ± 3.8	39 ± 0.9
<b>COPD</b>	<b>Control</b>	9.94 ± 0.6	71 ± 1.6	89 ± 1.6	45 ± 2.2
	<b>PECO</b>	10.37 ± 0.54	72 ± 1.6	90 ± 1.9	44 ± 2.3



**Table 3.**

	<b>COPD normocapnic</b>	<b>COPD hypercapnic</b>
<b>N</b>	9 (5 male)	9 (5 male)
<b>Age</b>	64.3 ± 6.1	67.6 ± 5.1
<b>Height (cm)</b>	160.1 ± 3.8	164.6 ± 3.2
<b>Weight (Kg)</b>	76.8 ± 3.7	74.6 ± 3.1
<b>Handgrip Force (N)</b>	239 ± 56	250 ± 51
<b>Resting SBP (mmHg)</b>	116 ± 4.6	121 ± 3.9
<b>Resting DBP (mmHg)</b>	77 ± 2.6	74 ± 1.9
<b>Resting MAP (mmHg)</b>	91 ± 3.2	89 ± 3.6
<b>Resting HR (bpm)</b>	71 ± 1.6	67 ± 1.3
<b>FEV<sub>1</sub> (L)</b>	1 ± 0.2	1.13 ± 0.3
<b>FEV<sub>1</sub> (% predicted)</b>	43.5 ± 5.1	40.5 ± 6.4
<b>FVC (L)</b>	2.24 ± 0.7	2.45 ± 0.8
<b>FEV<sub>1</sub>/FVC (%)</b>	44 ± 5.1	47 ± 4.3
<b>PaO<sub>2</sub> (mmHg)</b>	71.6 ± 5.3	63.3 ± 6.4*
<b>PaCO<sub>2</sub> (mmHg)</b>	37.7 ± 3.3	49.2 ± 2.7*
<b>pH</b>	7.41 ± 0.04	7.4 ± 0.03
<b>HCO<sub>3</sub> (mEq.L<sup>-1</sup>)</b>	25.3 ± 1.4	30.2 ± 1.6*

**Table 4.**

	<b>V (l.min<sup>-1</sup>)</b>	<b>HR (beats.min<sup>-1</sup>)</b>	<b>MAP (mmHg)</b>	<b>P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>
<b>Normocapnia</b>	9.9 ± 0.9	70 ± 1.6	92 ± 3.4	37 ± 1.7
<b>Hypercapnia</b>	10.8 ± 0.8	74 ± 1.2	89 ± 1.6	51 ± 2.3*

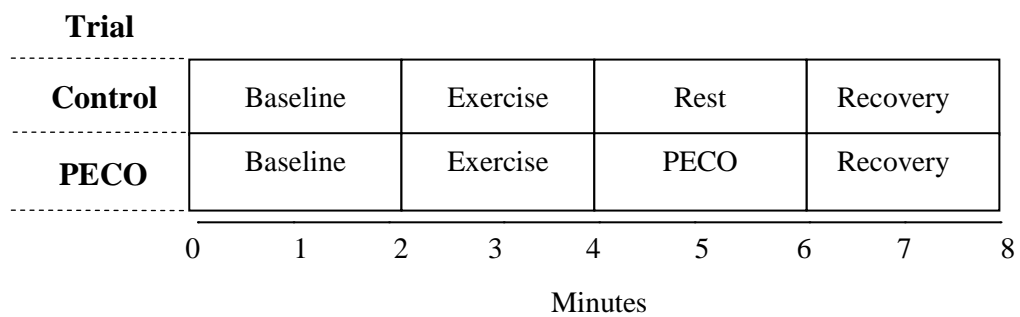
### Figure legends.

Figure 1. Schematic diagram of the 8 minute protocol for the Control and PECO trials.

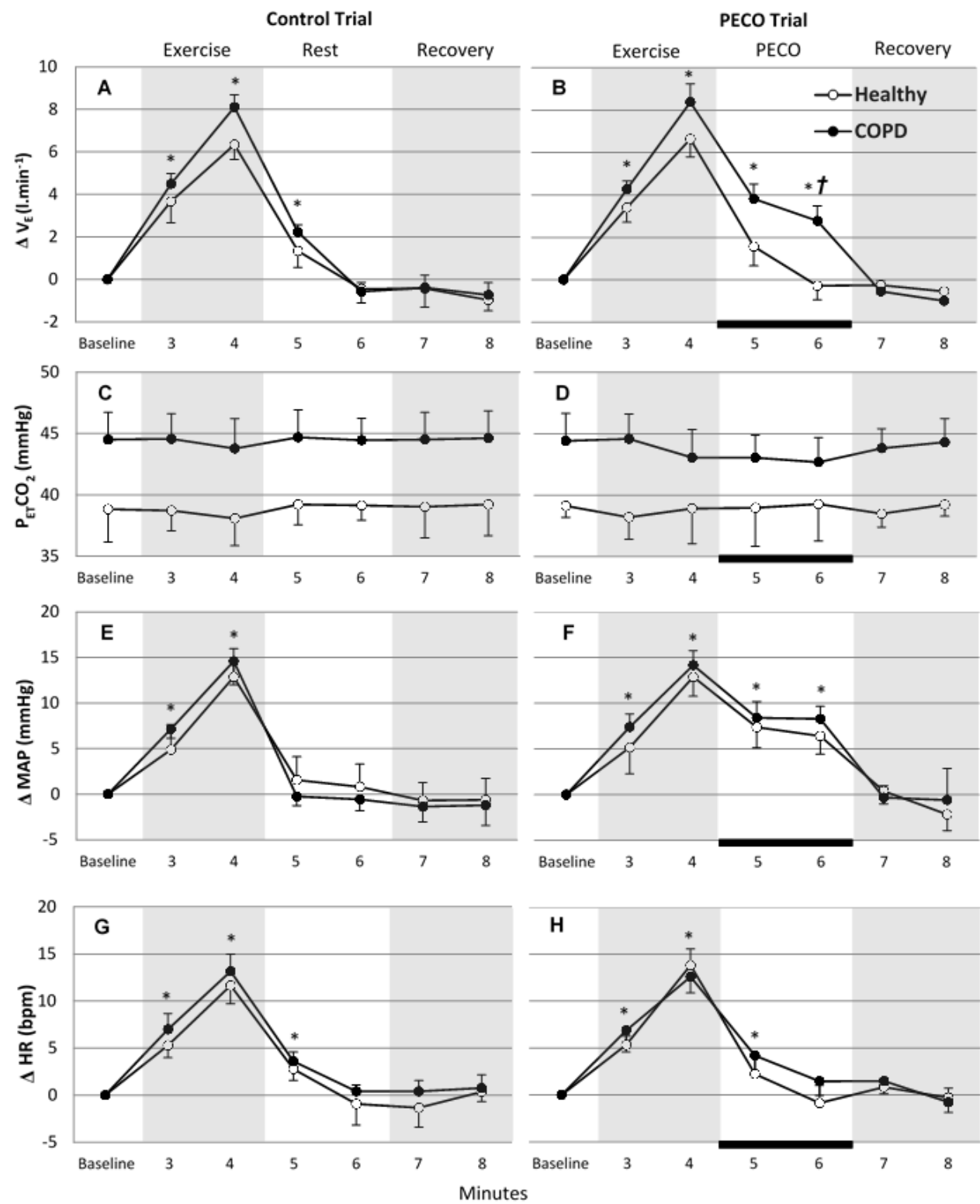
Figure 2. Change in V, MAP and HR from baseline, and  $P_{ET}CO_2$  during each minute of the control trial and PECO trial in COPD patients and healthy participants. The black bar indicates the period of circulatory occlusion. \* Significant difference from Baseline value ( $P<0.05$ ). † Significant difference between COPD patients and healthy participants.

Figure 3. Change in V, MAP and HR from baseline, and  $P_{ET}CO_2$  during each minute of the PECO trials in normocapnic and hypercapnic COPD patients. The black bar indicates the period of circulatory occlusion. \* Significant difference from Baseline value ( $P<0.05$ ). † Significant difference between normocapnic and hypercapnic groups.

**Figure 1.**



**Figure 2.**



**Figure 3.**

